The method comprises four steps: (i) amplifying a region of DNA comprising a polymorphic locus in the sample; (ii) labeling the amplified DNA products; (iii) hybridizing the labeled amplified DNA products to a probe on a solid support; and (iv) detecting the hybridized labeled amplified DNA products. The amplification is carried out using a pair of primers. The first primer terminates at its 3' end at the polymorphic locus and comprises a 3' portion which is complementary to the region of DNA and a 5' portion which is identical in sequence to all or part of the probe on the solid support and not complementary to the region of DNA. The step of amplifying produces a first strand and a second strand. The first strand comprises a portion identical to all or part of the probe, and the second strand comprises a 5' portion complementary to all or part of the probe. Claim 23 is directed to a method to prepare samples for analysis to determine a nucleotide at a polymorphic locus in a nucleic acid sample. The method recites the steps of amplifying, labeling, and hybridizing as in claim 1, but does not recite detecting.

The Patent Office has the burden of establishing a *prima facie* case of obviousness. (MPEP § 2142.) To establish *prima facie* obviousness of a claimed invention there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *In re Fine*, 837 F.2d 1071 (Fed. Cir. 1988); *In re Jones*, 958 F.2d 347 (Fed. Cir. 1992). It is respectfully submitted that the Patent Office has failed to establish motivation for combining the teachings of Vary and Lane, and thus fails to meet its burden of establishing a *prima facie* case of obviousness.

Vary teaches a method of detecting a target nucleotide sequence in the nucleic acids of a biological sample. The nucleic acids of the sample are contacted with a probe polynucleotide which anneals to a target nucleotide sequence. The probe polynucleotide is extended using

labeled nucleotides. The extended probe polynucleotide is detected. (Column 1, line 57 through column 2, lines 3-6.) Vary also teaches that the probe polynucleotide can be extended on an immobilized support. (Column 4, lines 14-17.) If extension occurs on an immobilized support, the probe polynucleotide comprises a 3' sequence complementary to the target nucleotide sequence and a 5' sequence complementary to a binding segment present in an immobilized polynucleotide attached to a support. (Column 7, lines 23-49; Figure 3.)

Vary does not teach or suggest at least two elements of the rejected claims. Vary does not teach a primer comprising a "5' portion which is identical in sequence to all or part of a probe on a solid support and not complementary to the region of DNA." (Emphasis added.) Vary teaches a probe polynucleotide comprising a 5' sequence complementary to an immobilized polynucleotide. (Column 7, lines 23-49; Figure 3.) Vary also does not teach a step of amplifying with a primer pair. Vary teaches single-strand extension of the probe polynucleotide. This step is neither amplifying nor does it employ a primer pair. The Patent Office Action concedes that these elements are not taught by Vary. "Vary et al. do not teach the comprising a primer pair wherein the first primer comprises a 5' portion which is identical in sequence to all of a probe on a solid support." (Paper 16, page 3, lines 7-9.) The Patent Office urges, however, that Vary's teaching could have been modified by Lane's teaching to correct these deficiencies.

Lane teaches detection of nucleic acid analytes in samples. In one of Lane's methods, a nucleic acid analyte already bound to a probe on a solid support is amplified with a primer pair. One primer of the pair comprises a 5' sequence identical to the probe. (Column 6, lines 31-41.)

The Office Action asserts that one of ordinary skill in the art would have been motivated to modify the teachings of Vary with Lane's teaching to arrive at the first element missing in

Vary, a primer comprising "a 5' portion which is identical in sequence to all or part of a probe on a solid support and not complementary to the region of DNA." The Office Action asserts:

> It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the 5' portion of the primer being complementary to the capture-probe in the method of Vary et al. with the 5' portion being identical to the capture probe as taught by Lane et al. to thereby immobilize the sense strand for the expected benefit [of] detecting the presence of a polymorphism in the coding strand.

Paper 16, page 3, lines 18-23.

The Patent Office has not explained its assertion that detecting polymorphisms in the "coding strand" is beneficial. One strand of the target DNA provides exactly the same amount of information as the other. The information provided to one of ordinary skill in the art in each strand is the same. Thus, to detect polymorphisms in the coding strand of target DNA would have provided no additional benefit over Vary's method. Vary taught that his methods are useful for identifying polymorphisms. Vary taught:

> In some forms, the 3'-terminal nucleotide of the probe polynucleotide is selected to form a matched pair with some sample strands, but a mismatched pair with other sample strands. In such cases, if the primer dependent enzyme used for extension is one lacking 3'-exonuclease activity, then only those hybrids forming such a matched pair will be extended and subsequently determined.

Lines 9-16 of the abstract. See also Figure 4 and column 12, line 19 through column 14, line 4. Thus there would have been no benefit to combining Vary with Lane to arrive at a primer that comprises "a 5' portion which is identical in sequence to all or part of a probe on a solid support and not complementary to the region of DNA."

The Office Action asserts that one of skill in the art would have been motivated to modify Vary's teaching with Lane's teaching to arrive at the second element missing from Vary, *i.e.*,

amplification using a primer pair. The Office Action urges that one of ordinary skill in the art would have been motivated to make this modification "to thereby more accurately determine the presence of polymorphic locus by analyzing the presence and quantity of both the first and second strands." Vary, however, provides no teaching or suggestion that indicates that more accurate determination is desired to determine the presence of a polymorphic locus. In fact, Vary teaches that the presence of a polymorphic locus is accurately determined using his method with single strand extension in Example 3 ("Base Specific Detection of Analyte DNA Sequences") and Example 4 ("Discrimination of E. Coli and S. Typhimurium 16S Ribosomal RNAs at the Position of a Single Base Mismatch.") (Column 12, line 15 through column 14, line 4.) Lane also does not teach that amplification provides more accurate determination than primer extension. Motivation to combine references for this reason is thus not found in the references themselves. The Patent Office provides no evidence that amplification would provide more accurate determination than primer extension. Applicants respectfully request that the Patent Office provide evidence in support of its assertion. "When a rejection is based on facts within the personal knowledge of the examiner, the data should be stated as specifically as possible, the facts must be supported, when called for by the applicant, by an affidavit from the examiner." MPEP § 2144.03 citing 37 CFR § 1.104(d)(2).

The Patent Office also asserts that one of ordinary skill in the art would have been motivated to substitute a double stranded amplification reaction for Vary's single strand extension reaction because Lane teaches that "the amplified products are restrained within a localized foci to thereby facilitate product detection." (Paper 16, page 8, lines 21-22.) Vary teaches that the single-strand extension products of his method are also restrained and detected on a solid support. Thus this reason provides no additional benefit over Vary. Vary teaches that

it is preferred to "perform a separation after the elongation step that normally involves the elongated hybrid being immobilized (if it is not already on a solid phase)." (Column 4, lines 15-17.) Vary further teaches that "detection can proceed in a conventional fashion, either on the solid phase or other wise." (Column 4, lines 54-56.) Thus there would have been no motivation for one of skill in the art to modify the teachings of Vary with Lane's. One of skill in the art would not have been motivated to change Vary's single strand extension reaction to a double stranded amplification reaction. Withdrawal of this rejection to claims 1 and 28 is respectfully requested.

The remaining claims (2, 4, 5, 7-9, 11-14, 16, 24, 26, 27, 29-31, 33-36, and 38) of the rejected set are dependent from either claim 1 or claim 28 and recite additional elements. The Office Action states that either Vary or Lane provides teachings of those additional elements which, when combined with the teachings discussed above, render the dependent claims obvious. Without admitting to the appropriateness of those teachings in rejecting the dependent claims as discussed in the Office Action, Applicants respectfully submit that the dependent claims are not obvious for the same reasons as independent claims 1 and 28.

Therefore, for the reasons discussed above, the withdrawal of this rejection is respectfully requested.

The Rejection of Claims 3, 10, 25, and 32 Under 35 U.S.C. § 103(a)

Claims 3, 10, 25, and 32 are rejected as obvious over Vary et al. (U.S. Patent No. 4,851,331) in view of Lane et al. (U. S. Patent No. 6,165,714), and Hames et al. (Nucleic Acid Hybridization: a practical approach, 1988, pages 35, 36, and 42-44), and Lapidus et al. (U.S. Patent No. 5,670,325). This rejection is respectfully traversed.

Claims 3 and 25 are dependent on claims 1 and 23, respectively, and further recite a step of labeling which is performed by a terminal transferase reaction. Claims 10 and 32 are dependent on claims 1 and 23 and require that the label is fluorescent and further recite the steps of optically detecting the fluorescent label on the solid support and determining the ratio of different allelic forms of the polymorphic locus from the relative amounts of label.

The Office Action concedes that Vary and Lane do not teach the use of terminal transferase for labeling as recited in claims 3 and 25. Hames is cited as teaching the use of terminal transferase to label the 3' end of DNA molecules with a single radiolabeled nucleotide. However, as discussed above, there would have been no motivation to combine the teachings of Vary with those of Lane to arrive at a primer having "a 5' portion which is identical to a portion of a probe on a solid support and not complementary to the region of DNA" as required by the subject claims. There also would have been no motivation to modify the method of Vary with Lane to use double-stranded DNA amplification in place of single-stranded DNA extension. Hames does not contribute any teaching that would motivate one of ordinary skill in the art to modify Vary with Lane and arrive at either of these elements of claims 1 or 28. Hames merely teaches scientific protocols that are used to perform terminal transferase reactions.

The Office Action further cites Lapidus as teaching the comparison of amounts of fluorescent label to determine whether an individual is heterozygous or homozygous as recited in claims 10 and 32. Lapidus, however, does not supply the missing motivation needed to combine Vary and Lane to use a first primer comprising "a 5' portion which is identical to a portion of a probe on a solid support and not complementary to the region of DNA." Rather, Lapidus teaches that polymorphisms are detected with an oligonucleotide "that is complementary to a portion of the region of [a] single-base polymorphism, said portion ending at the nucleotide that is

immediately 3' to the polymorphic nucleotide." (Column 16, lines 37-40.) Thus Lapidus teaches oligonucleotides that only contain sequences complementary to target DNA. Lapidus would not motivate one of ordinary skill in the art to modify Vary with Lane to arrive at a primer comprising a 5' sequence not complementary to target nucleotide sequence and identical to a probe sequence. Lapidus also does not supply the missing motivation needed to modify the teachings of Vary with Lane's to include a double-stranded amplification step as recited in the rejected claims. To detect a polymorphism, Lapidus teaches that the oligonucleotide is extended by one nucleotide in a single-stranded extension reaction. "[T]he polymerase will add one ddNTP to the 3' end of the probe, the incorporated ddNTP being complementary to the nucleotide that exists at the single-base polymorphic site." (Column 17, lines 25-28.) Thus Lapidus, like Vary, teaches single-strand extension to identify a nucleotide present at a polymorphic locus. Lapidus provides no motivation to one of ordinary skill in the art to modify Vary's single-strand extension reaction.

Therefore, for the reasons discussed above, the withdrawal of this rejection is respectfully requested.

The Rejection of Claims 6 and 28 Under 35 U.S.C. § 103(a)

Claims 6 and 28 are rejected as obvious over Vary (U.S. Patent 4,851,331) et al. in view of Lane et al. (U. S. Patent 6,165,714) and Mullan (U.S. Patent 5,455,169). This rejection is respectfully traversed.

Claims 6 and 28 are dependent on claims 1 and 23 and further require that the nucleotide is enzymatically labeled. The Office Action cites Mullan for teaching the use of enzyme-labeled oligonucleotide probes which bind to immobilized sample DNA. Mullan, however, does not

supply the missing motivation needed to combine the teachings of Vary and Lane to use a first primer comprising "a 5' portion which is identical to a portion of a probe on a solid support and not complementary to the region of DNA."

Mullan teaches that mutations at codons 670 and 671 of human amyloid precursor protein 770 can be detected by PCR amplification. Mullan teaches that amplification is performed with "oligonucleotide primers such that one is allele-specific. The desired allele is efficiently amplified, while the other allele(s) is poorly amplified because it mismatches with a base at or near the 3' end of the allele-specific primer." (Column 10, lines 28-33.) Thus Mullan teaches that the oligonucleotides used to amplify the gene encoding the amyloid precursor protein are complementary to the polymorphic gene sequences. Mullan does not teach that the primers comprise a 5' sequence not complementary to the gene sequence and identical to a probe sequence. Mullan also fails to supply the missing motivation needed to combine the teachings of Vary with Lane to use double-stranded amplification in place of single-strand DNA extension. Mullan simply does not teach any reason that would have been motivated one of skill in the art to perform double-stranded DNA extension.

Therefore, the withdrawal of this rejection is respectfully requested.

The Rejection of Claims 15 and 37 Under 35 U.S.C. § 103(a)

Claims 15 and 37 are rejected as obvious over Vary et al. (U.S. Patent 4,851,331) in view of Lane et al. (U.S. Patent 6,165,714) and Lockhart et al. (U.S. Patent 5,556,752). This rejection is respectfully traversed.

Claims 15 and 37 are dependent on claims 1 and 23 and further recite that the solid support is a microtiter dish. The Office Action states that Lockhart teaches the use of "microtiter

dishes (i.e. a polystyrene support having depressed regions)." (Paper 16, page 13, lines 8-9.) Lockhart, however, does not supply the missing motivation needed to combine the teachings of Vary and Lane to arrive at the first primer comprising "a 5' portion which is identical to a portion of a probe on a solid support and not complementary to the region of DNA." Lockhart teaches high-density arrays of oligonucleotides that can be used for sequencing or screening for genetic mutations. (Column 18, lines 42-44.) Lockhart, however, does not supply the missing motivation to modify the teachings of Vary with the teachings of Lane to use the claimed primer and the double-stranded DNA amplification. Lockhart does not supply the missing motivation to combine the teachings of the two primary references.

Therefore, the withdrawal of this rejection is respectfully requested.

Allowance of all pending claims is respectfully requested.

Respectfully submitted,

Date: May 24, 2002

By: Michelle L. Holmes-Son

Michelle L. Holmes-Son
Registration No. 47,660

Banner & Witcoff, Ltd.
1001 G Street, N.W., Eleventh Floor
Washington, D.C. 20001-4597
(202) 508-9100